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REPRODUCTIVE EFFECTS OF ALTERNATE DISINFECTANTS AND THEIR BY-PRODUCTS

by

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16. ABSTRACT
This is a review article of the reproductive effects of alternate disinfectants and their by-products. The available literature is surveyed concerning studies of chlorine, chloramine, chlorine dioxide, chlorite, and chlorate. A more detailed discussion is given of reproductive studies of chlorite in the rat.

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INTRODUCTION

There is evidence that the fertility of the U.S. population is declining. Infertility will be experienced by approximately 15 percent of the U.S. population of childbearing age[1]. If we consider three studies of selected fertile male populations conducted between 1938 and 1951, [2-4] the estimated median sperm concentration values ranged from 85 to 120 million sperm/ml. In comparison, the median sperm concentration for three study populations reported in the 1970's [5-7] ranged from 38 to 65 million/ml. While 44 percent of MacLeod and Gold's[4] 1951 study population of 1000 fertile men had sperm concentrations in excess of 100 million/ml, only 21 percent of Zukerman's[5] 1977 population of 4122 prevasectomy patients had sperm counts in this upper range. It should be noted that the median counts observed in the more recent studies approach the value (40 million) which MacLeod suggested was the threshold of infertility in 1951.

It is against this background that we must consider the potential reproductive effects of life-time exposure to chlorine or proposed alternative water disinfectants and their byproducts. The populations potentially at risk include not only the male and female populations of childbearing age, but also those perinatally exposed. The effects on the perinatal population may be immediate, resulting in birth defects or increased perinatal mortality or morbidity. The reproductive sequelae may not be immediately apparent

in that the germ cells may be affected, altering the fertility of the F_1, and/or succeeding generations. The perinatal population may be at increased risk not only because of the immaturity of fetal or newborn enzyme systems, but also because infants consume approximately three times more liquid per pound body weight than do adults[8]. This increased fluid intake relative to body weight would maximally expose the perinatal group to the products of water disinfection.

Despite the widespread and long-term use of chlorine as a disinfectant for drinking water, there are limited data in the literature with regard to its reproductive or other health effects. With increasing awareness of the potential health effects of chlorination by-products such as the trihalomethanes and haloacetonitriles, alternative water disinfectants have been proposed. Chlorine dioxide, ozone, and chloramine are among the alternative disinfectants under consideration. The reproductive effects of these chemicals and their degradation products such as chlorate, chlorite, and hypochlorite are also largely undetermined.

A review of the reproductive and perinatal effects of chlorine, chlorine dioxide (ClO_2), chlorite (ClO_2), chlorate (ClO_3), and monochloramine (NH_2Cl) will be presented. In addition, the results of a study from our laboratory on the reproductive effects of sodium chlorite administration in the drinking water of Long-Evans rats will be discussed. The effects of chlorite on sperm parameters have not been reported previously.

HUMAN STUDIES

The available data on the health effects of water disinfection in man are extremely limited. Michael et al[9] reported no significant adverse health effects in a human population exposed to 5 ppm chlorine dioxide (ClO_2) in the drinking water for three months. Decreases in hemoglobin, hematocrit, and red blood cell count were seen in one case of a glucose-6-phosphate (G-6-P) deficient diabetic individual who was considered high risk for oxidant stress. A causal relationship between ClO_2 exposure and hematologic changes in this individual could not be established, but the potential for hematologic changes resulting from ClO_2 disinfection has implications for the newborn population which is also at increased risk for oxidant stress. The possibility of perinatal susceptibility was examined by Tuthill, et al [10] and reported in 1982. Tuthill's epidemiologic historic record study examined the mortality and morbidity experience of newborns in two contiguous communities, Holyoke and Chicopee, MA. Holyoke used chlorine and Chicopee used high levels of chlorine dioxide in their water supplies during 1945, the year under study. Tuthill reported a statistically significant positive association between chlorine dioxide exposure and a diagnosis of prematurity and increased weight loss after birth. No differences in birth defects or mortality were observed. One significant question must be raised which cannot be answered by the Tuthill study. Was the observed change in perinatal morbidity a result of ClO_2-based water disinfection or was the increased morbidity a response to the high levels of contamination in the water supply which necessitated the use of high levels of ClO_2 in lieu of chlorine?

ANIMAL STUDIES

In 1982 Abdel-Rahmen and coworkers[11] reported preliminary data on the effects of chlorine and monochloramine in the developing rat fetus. These teratologic evaluations revealed little or no effect of either chemical at levels up to 100 ppm. A slight increase in skeletal variants was noted following chlorine exposure, but the number of dams treated and fetuses examined was insufficient for both chemicals studied.

The teratogenicity of chlorine dioxide and its degradation products was studied by Suh, Abdel-Rahman, and Bull[12]. As in the previously described study, the number of litters per dose group was small (6-9 per group). No significant differences were observed in maternal toxicity, litter size, fetal weight, or gross, visceral, or skeletal anomalies following exposure to ClO_2 , ClO_2^- and ClO_3^- .

Couri et al[13] examined the teratogenic potential of sodium chlorite exposure by gavage, intraperitoneal (IP) injection, or in the drinking water of Sprague-Dawley rats. Again, the number of litters per group was small (4-13 dams/group). Decreases in maternal food and water consumption and body weight were observed in all treatment groups except the dose group receiving 0.1% chlorite in the drinking water. IP injection with 20 or 50 mg/kg daily and gavaging with 200 mg/kg chlorite resulted in vaginal and urethral bleeding, and death. Drinking 2% sodium chlorite in water, or injection of 10 or 20 mg/kg resulted in increased stillbirths and resorption sites, probably reflecting maternal toxicity. No fetal malformations were found with any treatment regimen.

The effect of chlorite administration during pregnancy was examined in two strains of mice, A/J and C57L/J, by Moore and Calabrese[14]. The primary focus of this study was on G-6-P dehydrogenase activity and hematologic parameters. Conception rate and litters were examined for A/J mice. A statistically significant ($p<0.05$) decrease was found in the average weight of pups at weaning and in the birth-to-weaning growth rate. Birth weight, litter size, perinatal survival, gestation length, and water consumption were not different for those mice receiving 100 ppm chlorite when compared with controls.

Reproductive parameters other than conception rate and perinatal survival, growth, and malformation rates have not been previously reported in the literature. In their study of the toxicologic effects of ClO_2, ClO_2, and ClO_3 Abdel-Rahman et al[15] included a study of tritiated-thymidine incorporation into the nuclei of liver, kidney, intestinal mucosa, and testes. Their studies suggested the possibility of increased DNA turnover in the intestinal mucosa and decreased DNA synthesis in several organs. Decreased ^3H-thymidine incorporation was observed in the testes following treatment with 10 or 100 ppm ClO_2, ClO_2 , or ClO_3 in the drinking water for three months. The biological significane is difficult to interpret, but the suggestion of decreased DNA synthesis in the testis is of interest with regard to potential effects on the reproductive system.

CURRENT STUDIES

We have examined the effects of administration of 0, 1, 10, 100, or 500 ppm sodium chlorite in the drinking water of Long-Evans rats in a series of three experiments.

Methods

Long-Evans rats, 4-6 weeks of age, were obtained from Charles River Breeding Laboratories (Portage, MI) and were housed two per cage in polycarbonate cages with Absorb-Dri® bedding. The animal room was maintained at 40-60 percent humidity and 69-75°F, and with room lights on from 6 am to 6 pm. Animals were given ad libitum access to Purina Certified Rodent Chow 5002 and deionized water or deionized water containing sodium chlorite as drinking water. In the first study, the effect of chlorite on reproductive outcome was evaluated. Twelve male rats per dose group receive 0, 1, 10, or 100 ppm chlorite for 56 days prior to breeding and throughout the 10-day breeding period. Twenty-four female rats per dose group received the same dose levels of chlorite for 14 days prior to breeding, and throughout breeding, gestation, and lactation until the pups were weaned on Day 21. Water consumption was recorded three times per week. During the 10-day breeding period, animals were housed 1 male:2 females. Females were check for the presence of sperm in a lavage smear prior to 9:30 am each day. Females observed to be sperm positive (gestation day 0) were individually housed. The day of parturition was designated lactation day 0.

Following the breeding period, males were bled for a complete blood count, killed by pentobarbitol overdose, and given a complete gross necropsy. The reproductive tract was removed, weighed, and, except for the right cauda epididymis, preserved for histopathologic evaluation. The right cauda epididymis was weighed and finely minced in 10 ml of phosphate buffered saline (PBS) with 0.1 percent glucose (pH 7.4) maintained at 37°C. The sperm suspension, and all slides, coverslips, solutions, and pipettes were maintained at 37°C. One to two drops of sperm suspension were placed on a microscope slide, coverslipped, and observed using phase-contrast microscopy. A modification of Katz and Overstreet's[16] videomicrography method was used to videotape and evaluate a minimum of ten 10-second fields at 160X magnification and ten 10-second fields at 400X magnification. An aliquot of the sperm suspension was heat-killed and used for determining sperm counts. A 0.5 ml aliquot was stained with aqueous eosin Y, and used to prepare 4-6 slides per animal for sperm head morphology evaluation according to the method of Wyrobek and Bruce[17]. The videotapes were later evaluated for percent sperm motility (10 fields) and sperm drive range (µm/sec: 5 sperm/field; 10 fields).

Dams were observed for conception rate, length of gestation, and body weight gain. At necropsy on lactation day 21, dams were bled for complete blocd count, killed by pentobarbitol overdose, and given a complete gross necropsy. The reproductive tract was taken, weighed, and preserved for histopathologic evaluation.

Litters were evaluated for litter size, perinatal survival, day of eye opening, day of vaginal patency, body weight gain, and gross external abnormalities. At necropsy on lactation day 21, pups were bled for complete blocd counts. The reproductive tract was weighed and preserved.

In experiment 2, groups of 12 male rats were given 0, 100, or 500 ppm sodium chlorite in deionized water as drinking water for 72-76 days. At necropsy, animals were bled for complete blood counts and methemoglobin determinations. Sperm suspensions were prepared and sperm count, percent motility, drive range (μm/sec), and sperm morphology abnormalities were determined as described above.

The third study replicated the second experiment, but groups of 12 male rats received 0, 10, or 100 ppm sodium chlorite in deionized water as drinking water.

Results

The body weights for adult animals in the three studies were unaffected by chlorite exposure. Water consumption was decreased by 28% among 500 ppm dose group animals.

No effect of chlorite exposure were noted when general reproductive ability was evaluated (Table 1). Conception rates varied between 67% and 96%, but did not differ in a dose-dependent manner. Perinatal survival was comparable for all groups as were litter size and pup body weight. The median day of eye opening (day 16), indicative of general pup maturity, and the day of vaginal patency (day 32), indicative of sexual maturation, were the same for all groups.

Clinical pathology, including methemoglobin levels, and histopathologic evaluation of the reproductive tract revealed no chlorite-induced abnormalities. Organ weights and organ-to-body weight ratios also were unchanged in chlorite-exposed rats.

When sperm counts, sperm morphology, and videomicrographic records of sperm were analyzed, comparable results were observed for the three study replicates. Sperm count was unaltered by chlorite exposure (Figure 1). The percent of motile sperm, when sperm were counted as

motile if any movement was detected, was not statistically different between groups, but a slight trend toward decreasing motility was observed among rats exposed to 10 ppm chlorite or greater (Figure 2). Standard deviations were relatively large, with coefficients of variation ranging from 34% to 58%. For animals in the 100 ppm and 500 ppm dose groups, mean sperm drive range (μm/sec) was significantly reduced ($p<0.01$) (Figure 3). Sperm drive range dropped from 20.3 μm/sec for control animals to 13.4 μm/sec and 11.0 μm/sec for 100 ppm and 500 ppm dose group animals, respectively. Decreasing velocity (17.5 μm/sec) was also observed for 10 ppm dose group males, but the difference from control values was not statistically significant.

Sperm morphology was determined for 500 sperm per rat from eosin Y-stained morphology slides. Approximately 0.8% of sperm from control animals exhibited abnormal sperm morphology. Rats from the 1 and 10 ppm dose groups had comparable rates of abnormal sperm morphology, 0.6% and 0.9%, respectively. A significant ($p<0.001$) increase in abnormal sperm forms was observed among 100 and 500 ppm dose group rats. In the 100 ppm dose group 2.9% (N=36 rats) and 1.8% (N=12 rats) in the 500 ppm dose grouop exhibited abnormal sperm morphology (Figure 4). The lower percent of abnormal sperm observed for the 500 ppm dose group animals relative to the 100 ppm dose group males may be influenced by the fact that only 1/3 as many rats were evaluated in the former group as in the latter. The most commonly observed morphologic abnormalities were frayed tails, open hooks, and amorphous sperm heads (Figure 5).

Discussion

While the magnitude of changes in sperm parameters reported here is comparatively small, the rats in this study were exposed for

the duration of one spermatogenic cycle only. The human population would be exposed to chlorite in the water supply for a life-time exposure. As discussed by Amann and Howards[18], daily human sperm production is generally below that of other mammals, primarily resulting from a lower number of germ cells within the seminiferous tubules. While the incidence of abnormal sperm forms for the control group rats was 0.8 percent, as many as 20-40 percent of sperm forms may be abnormal in man[19].

Meistrich and Brown[20] mathematically estimated the increased relative risk of human infertility from alterations in semen characteristics. They found the increased risk following exposure to industrial and environmental polluants was dependent upon and inversely related to the sperm count of the exposed population.

Sperm morphology appears to be a useful indicator of chemical toxicity in rodents[21] and man[22]. Increased incidence of abnormal sperm forms and decreased count and motility have been associated with increased incidence of abortion, abnormal pregancies, and infertility[23-25]. Further studies are required to provide information to assess the potential impact of aqueous chlorite exposure on human reproduction performance, given man's possibly declining fertility and the potential for life-time exposure.

Table 1. Reproductive Performance of Long-Evans Rats Treated with 0, 1, 10, or 100 ppm Sodium Chlorite

	0 ppm	1 ppm	10 ppm	100 ppm
No. ♀ Bred	24	24	24	24
No. Sperm Pos.	23	24	22	22
No. Litters (D0)	23	16	22	21
No. Litters (D7)	23	16	22	21
No. Litters (D21)	23	16	22	21
$\bar{X}$ Litter Size	13	13	11	13
Mdn Day Eye Opening	16	16	16	16
Mdn Day Vag Opening	32	—	—	32

Reproductive Performance of Long-Evans Rats Treated with 0, 1, 10, or 100 ppm Sodium Chlorite

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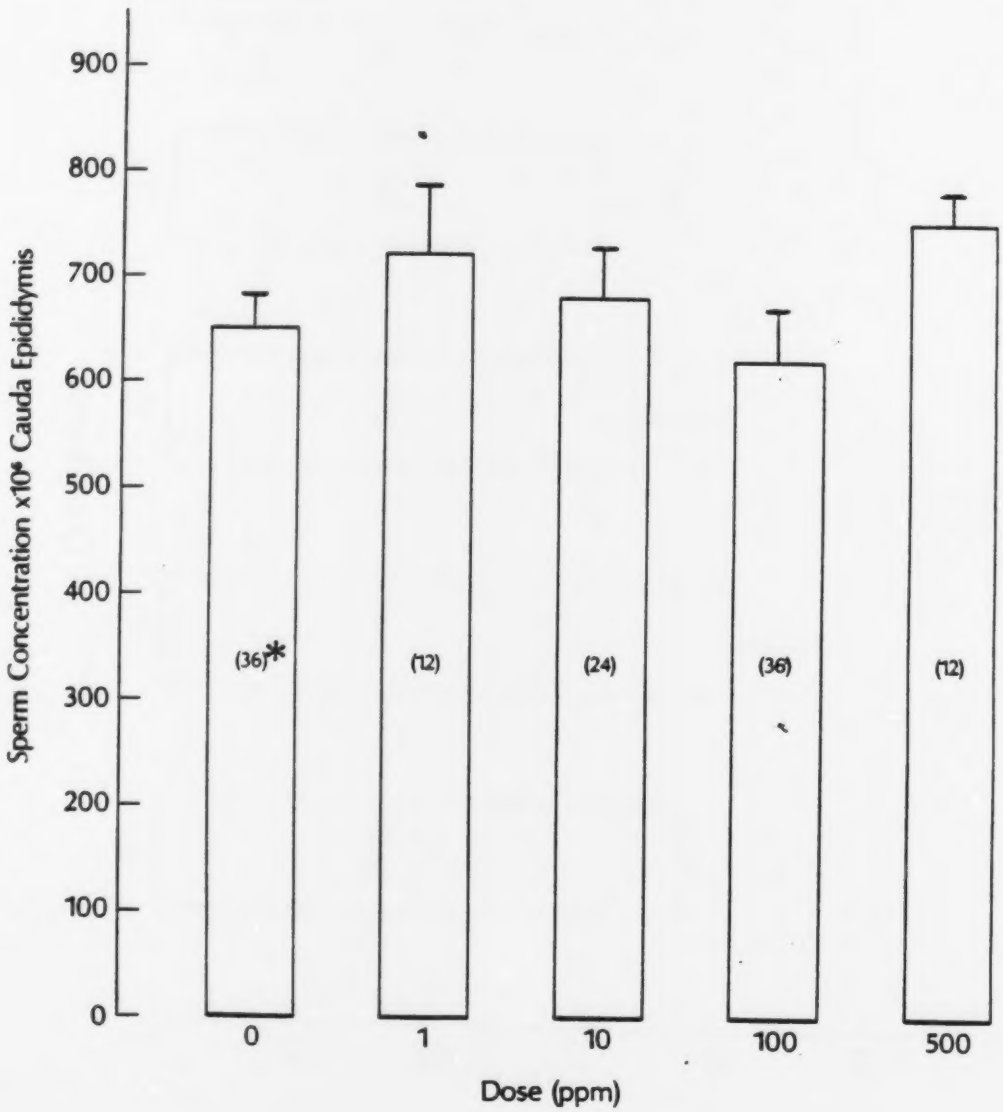
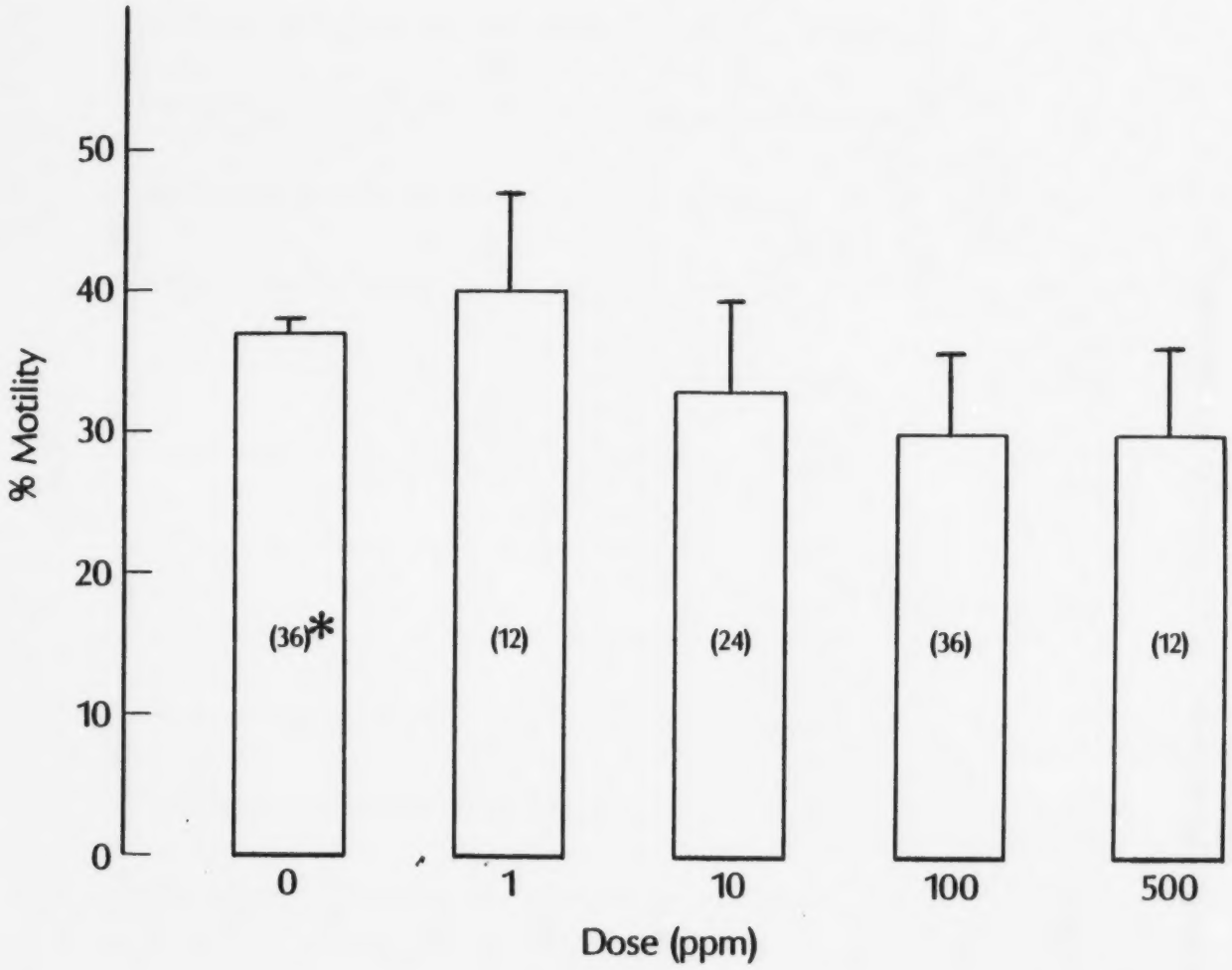
No. ♀ Bred	24	24	24	24
No. Sperm Pos.	23	24	22	22
No. Litters (D0)	23	16	22	21
No. Litters (D7)	23	16	22	21
No. Litters (D21)	23	16	22	21
$\bar{X}$ Litter Size	13	13	11	13
Mdn Day Eye Opening	16	16	16	16
Mdn Day Vag Opening	32	—	—	32

1. Sperm Concentration (x10⁶/g cauda epididymis)*



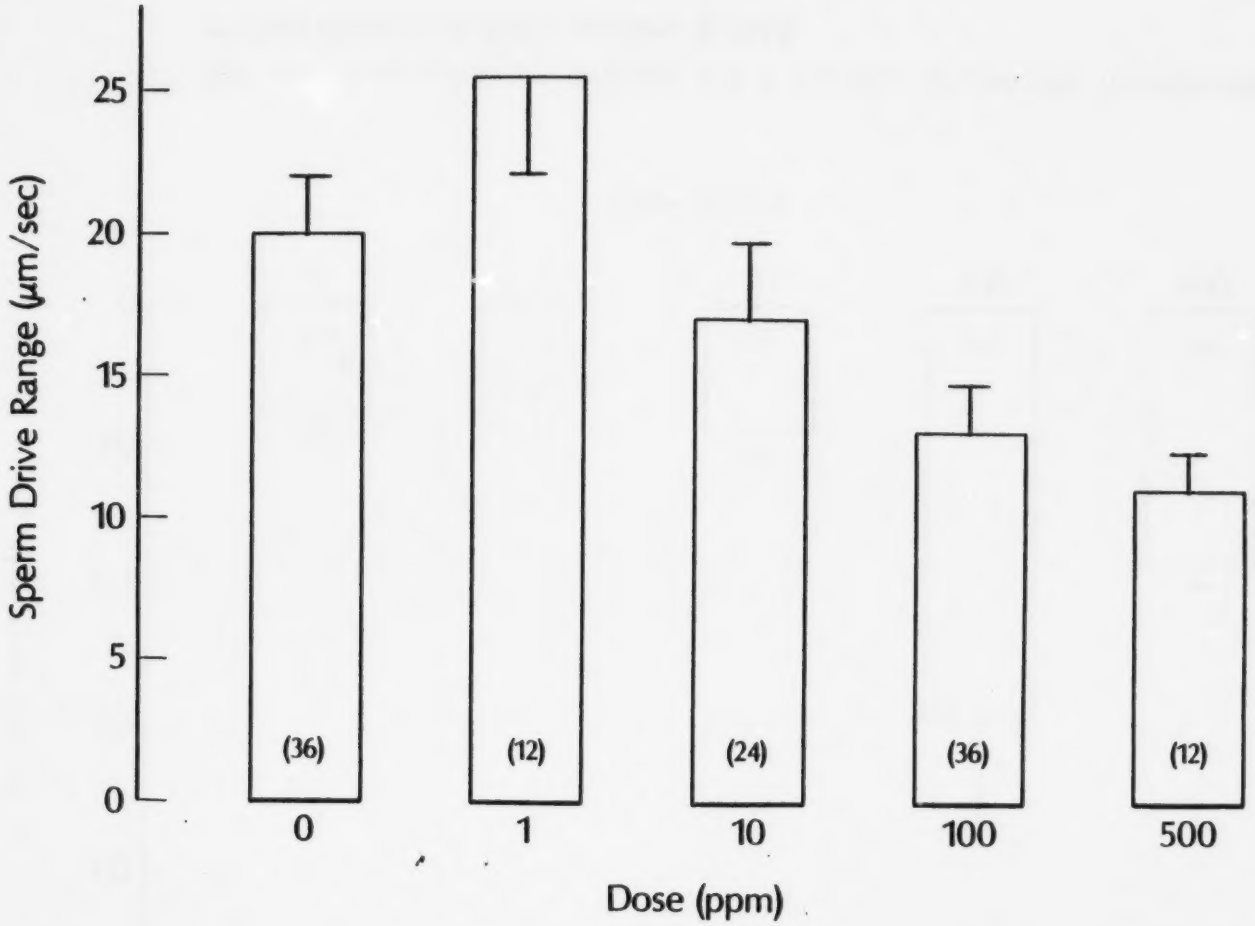
* (N) = Number of animals. Results are pooled data from three studies. ($\bar{x}$±SEM)

2. Percent Motile Sperm*



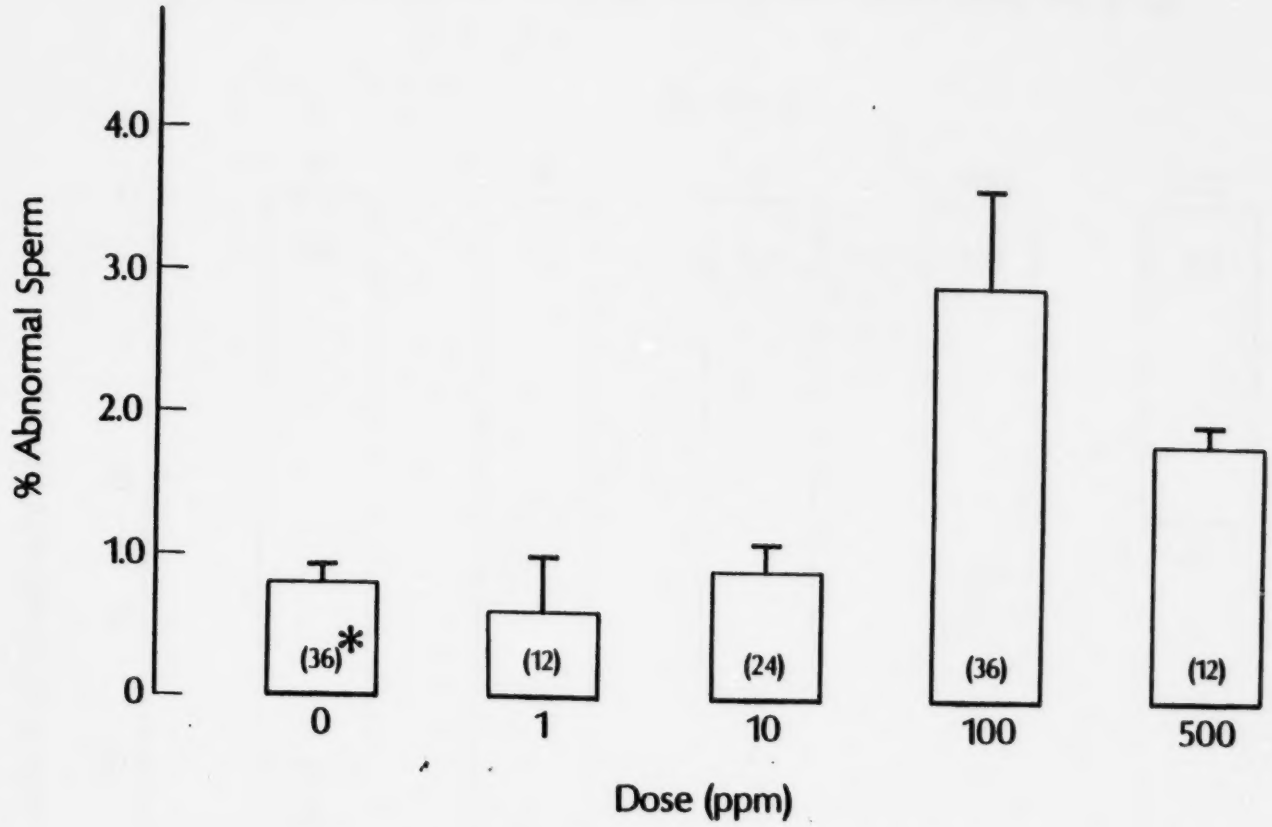
* 10 independent fields per study. (N) = number of animals. Results are pooled data from three studies. ($\bar{X}$±SEM)

3. Sperm Drive Range (μm/second) *



* 5 sperm per field for each of 10 fields (50 sperm total) per study. (N) = number of animals. Results are pooled data from three studies. ($\bar{x}$±SEM)

4. Percent Abnormal Sperm Morphology*



* 500 sperm counted per animal. (N) = number of animals. Results are pooled data from three studies. ($\bar{x}\pm SD$)

FIGURE 5. COMMONLY OBSERVED SPERM MORPHOLOGY. A. NORMAL B. OPEN HOOK C. AMORPHOUS D. AMORPHOUS WITH FRAYED TAIL

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